Effects of space flight on neutrophil functions in astronauts

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ABSTRACT

Neutrophil phagocytosis, oxidative burst, degranulation, and the expression of selected surface markers were studied in 25 astronauts following 4 space shuttle missions. Space flight duration ranged from 5 to 11 days. Blood specimens were obtained 10 days before launch, immediately after landing, and again at 3 days after landing. The number of neutrophils increased at landing by 85%. Phagocytosis of *Escherichia coli* (*E. coli*) and oxidative burst following the medium length (9 to 11 days) missions were lower than the control mean values. Whereas, following the short-duration (5 days) mission, these functions were unchanged from control values. No consistent changes in degranulation were observed following either short or medium length space missions. The expression of CD16, CD32, CD11a, CD11b, CD11c, L-selectin and CD36 were measured and found to be variable. Specifically, CD16 and CD32 did not correlate with the changes in oxidative burst. Mission duration appears to be a factor in phagocytic and oxidative functions.

INTRODUCTION

Space flight is a unique experience for humans. Remarkably healthy individuals live and work in relatively crowded and dangerous microgravity environments. Stresses associated with space flight, such as sleep deprivation, can impact astronaut health, and adverse effects resulting from such stress may increase as mission duration increases (1). Studies of space flight and the human immune response have shown changes in lymphocyte proliferation (2), phenotyping of peripheral blood mononuclear

cells (3-4), cytokine production (2, 5, 6), natural-killer cell cytotoxicity [2], cell-mediated immunity (2, 7), and other aspects (1).

Neutrophils are an important element of astronaut health, and previous studies have reported increases in the number of neutrophils of more than 100 percent following space flight (1). Stowe et al. (8) were the first to report changes in neutrophil functions following space flight by demonstrating alterations in neutrophil chemotaxis and adhesion to endothelial cells in astronauts following short-duration space flight.

With the International Space Station now a reality, a near continuous occupation with international crews is imminent. An understanding of the effects of space flight on the human immune response is essential to ensure the health, safety, and performance of the international crewmembers. No studies on the ability of neutrophils to phagocytize and kill bacteria have been conducted on astronauts. This study was undertaken to examine the basic functions of neutrophils necessary to meet invading microbial challenges.

MATERIALS AND METHODS

Subjects

Twenty-five astronauts from 4 space shuttle missions of 5 to 11 days duration were the subjects for this study. The 16 men and 9 women ranged in age from 38 to 77 years. A control group of nine healthy subjects was included for comparison studies. The 8 male and 1 female control subjects ranged in age from 42 to 56 years. The study was approved by the Johnson Space Center Institutional Review Board and informed consent was obtained from all subjects.

Specimen Preparation

Ten-milliliters of blood were collected in EDTA Vacutainer tubes (Becton Dickinson, Mountain View, CA) from each crewmember 10 days before flight (L-10), within 3 hours after landing (R+0), and again on the third day after landing (R+3). Due to restrictions in the availability of the astronauts blood samples, the blood samples available for this study were collected in EDTA. We have standardized these assays to be performed optimally in blood collected in EDTA and compared values to samples collected in heparin as an anticoagulant. Similarly, blood specimens were also collected from the control subjects in EDTA Vacutainer tubes at three time points simulating a 10-day space mission. The blood specimens were centrifuged at 400 g for 20 min, and the plasma was removed. The remaining blood cells were washed twice in DPBS-Glucose washing solution (Dulbecco's phosphate buffered saline without calcium and magnesium containing 5 mM glucose), and resuspended to the original volume in DPBS containing 133mg/L $CaCl_2.2H_2O$ and 100mg/L $MgCl_2.6H_2O$ and 5 mM glucose (Sigma-Aldrich, St.Louis, MO) and 20% (v/v final concentration) human AB serum (Biowhittaker, Walkersville, MD). This blood-cell preparation was then used to perform the neutrophil functional studies and the surface molecule phenotyping described below.

Phagocytosis

The phagocytic capability of neutrophils was determined using the Phagotest Kit (Orpegen, Heidelberg, Germany). Briefly, 0.2 ml of the blood-cell preparation was incubated for 30 min at 37°C with opsonised *E. coli* labeled with fluorescein

isothiocyanate (FITC). Non-specific binding was determined by incubating 0.2 ml of CP with opsonised *E. coli*-FITC at 4°C for 30 min. At the end of the incubation, all the tubes were transferred on ice to stop the reaction. The fluorescence of the surface bound *E. coli*-FITC was quenched by adding 0.2 ml of ice cold Trypan blue (provided in the kit) and immediately adding 3 ml of the Phagotest wash buffer (provided in the kit). The cells were centrifuged at 400 g for 10 min, the supernatant was removed and the residual cells were incubated for 10 min at room temperature with 4 ml of RBC lysing solution (provided in the kit). The cells were washed twice by centrifugation with the Phagotest wash buffer and resuspended in 0.5 ml of 1% paraformaldehyde in DPBS. O.1 ml of DNA staining solution (Propidium iodide-provided in the kit) was added to the cells before analysis on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) using the CellQuest™ software. A gate was set for the leukocytes on the FL2-fluorescence channel (575 nm emission). This gate differentiates between leukocytes and bacteria. Further gates were set on the neutrophils on the basis of their forward and side scatter. We collected 40,000 events in the neutrophil gate. Phagocytosis is reported here as the increase in the mean fluorescence intensity (MFI) per cell of the cells incubated at 37°C over the MFI of the cells incubated at 4°C, as calculated by the CellQuest™ program. The 'mean control' line is the mean of all the values from all the control subjects at all time points. Preflight values are from samples taken 10-days before flight, landing values are from samples within 3-hours of landing and postflight values are from samples taken 3-days after landing.

Oxidative Burst Capacity

Oxidative burst was determined using the Fc OxyBURST Green Assay reagent (Molecular Probes, Eugene, OR). Fc OxyBURST Green assay reagent consists of bovine serum albumin (BSA) covalently linked to dichlorodihydrofluorescein (H_2DCF) and complexed with purified rabbit polyclonal anti-BSA IgG antibodies to yield a multivalent particulate immune complex. When these immune complexes bind to the Fc receptors on the neutrophils, the nonfluorescent H_2DCF molecules are internalized within the phagovacuole and subsequently oxidized to green fluorescent dichlorofluorescein (DCF). The blood-cell preparation (0.4ml) was incubated with 200 µg/ml (final concentration) of the Fc OxyBURST Green reagent in a 37°C water bath for 10 min. Non- specific fluorescence was determined by incubating 0.2ml of the blood-cell preparation with Fc OxyBURST Green reagent at 4°C for 10 min. At the end of the incubation, 4 ml of FACSlysing solution (Becton Dickinson, San Jose, CA) was added to all the tubes that were then incubated in the dark for 10 min at room temperature. The samples were centrifuged at 400 g for 5 min to remove the FACSlysing solution. The residual cells were washed with DPBS-Glucose, centrifuged and resuspended in 0.5 ml of 1% paraformaldehyde in DPBS. Oxidative burst was determined from the increase in MFI at 37°C over the value at 4°C, as described above.

Degranulation

Neutrophil degranulation was determined by measuring elastase release using a multivalent immune complex, DQ-HIC, prepared as previously described (9) from BSA covalently linked to a BODIPY™ containing elastase substrate (Molecular Probes, Eugene, OR) and anti-BSA IgG. When exposed to elastase, the substrate is cleaved

and the resultant is highly fluorescent (ex. 488 nm, em. 530 nm). As described by Seetoo et al. (9), this cleavage is elastase-specific and occurs preferentially in the phagocytic vacuoles enclosing the DQ-HIC, permitting observation by flow cytometry. The blood-cell preparation (0.4ml) was incubated with 500µg/ml of the HIC-DQ complex at 37°C for 15 min. As a control for non-specific fluorescence, the reaction mixture was incubated on ice for 15 min. At the end of the incubation, 4 ml of FACSlysing solution was added to lyse the RBCs, and the mixture was incubated in the dark for 10 min at room temperature. The cells were washed with DPBS-glucose and the residual cells were resuspended in 0.5 ml of 1% paraformaldehyde in DPBS. Elastase release was evaluated from the increase in MFI at 37°C over that at 4°C, as described above.

Phenotyping for Surface Molecules

Phenotyping of the surface markers was achieved by incubating 0.2 ml of the blood-cell preparation for 20 min with 20 µl of each of the following antibodies coupled either to FITC or phycoerythrin (PE): anti-CD16, anti-CD11a, anti-CD11b, anti-CD11c, anti-Leu8, (Becton Dickinson), anti-CD32, and anti-CD36 (Coulter-Immunotech, Miami, FL). Antibody isotype controls coupled to the respective fluorochromes were used to measure non-specific binding. Red blood cells were lysed by incubating the cells with FACSlysing solution in the dark for 10 min at room temperature. The cells were washed with DPBS containing 1% Fetal Calf serum and 0.1% Sodium azide and centrifuged at 400 g for 10 min. The cells were resuspended in 0.5 ml 1% paraformaldehyde in DPBS, and the specific fluorescence was determined. The number of cells positive for each surface marker (corrected for non-specific binding) was evaluated, as was the

intensity of expression of each surface marker, as evaluated (corrected for non-specific binding) from the shift in MFI of the positive cells.

Statistical Analysis

For each of the types of measurement described in this section, analysis of variance (ANOVA) was used to assess: a) the overall effect of the flight by comparison of means for each mission to controls, b) the effect of flight phase (i.e. preflight, landing and postflight) on the mean response for each mission, and c) the extent of the interaction (i.e. the phase effect), and whether it differed significantly between each mission and the control subjects. In cases where the data did not satisfy ANOVA assumptions of a normal distribution and homoskedasticity within treatment groups, non-parametric tests (Mann- Whitney Rank Sum or Friedman's ANOVA on Ranks) were used as alternatives. Results were reported as statistically significant in cases where the P-value was found to be less than 0.05. We used the pattern of results over the different functions to point to a meaningful conclusion about the overall effect.

RESULTS

Neutrophil Number

Immediately after landing following 4 space shuttle missions, the number of neutrophils in 25 astronauts increased an average of 85% higher than the 10-day preflight values (data not shown). This marked increase in neutrophils resulted in a corresponding increase (50% average) in the total WBC counts at landing (data not shown). No association with age, gender, mission duration, or flight event was evident. It should be

noted that, since the functional assays are calculated on the effect of activation per cell, the number of cells being measured does not affect the functional assay values.

Phagocytosis

The ability of the neutrophils to engulf opsonised E.coli was used as an indicator of their phagocytic capacity (Fig. 1). While small variation among the individual control subjects (panel A) was evident; however, the variation in the phagocytic capacity of all individuals during the three sampling periods was not significant. Panels B,C,D, and E depict phagocytic capability of the neutrophils of the astronauts on space shuttle missions of 5, 9, 10, and 11-day duration, respectively. In contrast to control subjects, individual astronauts on all 4 space flights showed considerable variation during the three sampling periods (10-days before launch, at landing, and 3-days after landing) ($p<0.001$). Neutrophil phagocytosis from the 5-day mission, while variable, was very similar to the control subjects (mean of the three values of all 5 crewmembers of the 5-day mission was 963). The 3 longer missions showed considerable variation among the 20 crewmembers and among sampling times; however, some important trends were evident: First of all, the mean phagocytosis of all of the individual astronauts' values of the three longer missions were below the control mean of 975 ($p=0.001$ or <0.001), with the 9-day mission's mean being the lowest (334). The preflight values were particularly depressed. The postflight values of all three longer missions were consistently below the control mean ($p=0.001$ for 9- and 10-day missions, $p=0.002$ for 11-day mission). At landing, there was an increase in the phagocytic capacity of the astronauts' neutrophils when compared to either the preflight or postflight values except for two crewmembers

(#1 and 3 of the 10-day mission, Panel D), where that value was just slightly lower than preflight albeit higher than postflight. It should be noted that comparison of phagocytosis with measurements of oxidative burst and degranulation can only be qualitative since phagocytosis could have occurred via FcR or C3b and C3bi while the other two functions were measured with immune complexes which excite only via Fc receptors.

Oxidative Burst Capacity

The oxidative burst capacity of the neutrophils was estimated using the Fc OxyBurst Green assay reagent from Molecular Probes. The variation in oxidative burst among the control subjects over the three sampling periods was very small (Fig. 2). The inter-subject variation was also very small except for #8 and 9 whose values were about 33% higher than the rest of the controls. As was true for phagocytosis, the preflight mean values on all 4 missions were significantly lower than the control mean value ($p<0.001$, 0.003, 0.02, 0.002, respectively). However, the landing and postflight values in all 5 subjects of the 5-day mission were at or above the control mean. With few exceptions the oxidative burst capacities of the neutrophils of crewmembers on the 9, 10 and 11-day missions were consistently lower than the controls. The 11-day mission showed the most diminished oxidative burst mean value (135) indicating a length of mission effect (Fig. 2).

Degranulation

Degranulation of the neutrophils was measured by estimating the release of elastase from the granules into the phagocytic vacuoles (Fig. 3) and is identified by DQ™, the substrate for elastase that becomes fluorescent after the enzyme-substrate reaction. Again, there is little variation among the control subjects (Panel A) with the exception of control subject #6 whose values were 7 fold higher than the other control subjects. The mean for the control subjects was calculated by omitting the values for control subject #6. There were small variations in the degranulation capacity of the neutrophils from the control subjects during the 3 sampling periods. The 5, 10, and 11-day missions (Panels B, D, and E) exhibited degranulation values similar to the mean of the control subjects. However, degranulation of the neutrophils of the crewmembers of the 9-day mission (mean = 811) (Panel C) was higher than the control subjects.

Surface and Adhesion Molecules

CD32 (FcRII): CD32 represents a low-affinity binding site for the Fc end of IgG. At landing, there was a decrease ($p=0.011$) in the expression of CD32 on the neutrophils of crewmembers of the 5-day mission whereas there was a significant increase ($p<0.001$) for the crewmembers of the 10-day mission (Fig. 4). The increase in CD32 expression observed at landing among the crewmembers of the 11-day mission was not statistically significant. At R+3, the levels of CD32 recovered back to near or more than preflight levels among crewmembers of the 5-day mission, but fell down sharply on the neutrophils of crewmembers of the 10-day mission ($p<0.014$).

CD16 (Fc RIII): CD16 represents an intermediate affinity binding site for the Fc end of IgG. The expression of CD16 on the neutrophils of control subjects (Fig. 4) did

not change significantly over the 3 different time points of sample collection. But there was a significant variation in the expression of CD16 on the neutrophils of all the crewmembers ($p=0.03$ for 5-day mission, $p=0.001$ for 9-day mission, $p=0.012$ for 10-day mission and $p=0.003$ for the 11-day mission).

CD11a (LFA-1): CD11a or the Leukocyte function antigen, a ß2-integrin, is important in leukocyte-endothelial interactions. The number of neutrophils of control subjects (Fig. 4) expressing CD11a did not change significantly over the 3 different time points of sample collection. In contrast, the neutrophils of the crew of the 5-day mission had very low expression of CD11a compared to the control subjects (Fig. 6). Among the crewmembers of the 9-day mission, the percent of neutrophils expressing CD11a was in the normal range at 10 days before flight and at landing, but these levels fell drastically 3 days postflight. 5/7 of the crewmembers of the 11-day had very low numbers (1-6%) of neutrophils expressing CD11a; they recovered to normal values at landing, but declined sharply 3 days postflight. Five out of six crewmembers of the 11-day mission also had a very low percentage (1-13%) of neutrophils expressing CD11a before flight. But at landing and 3 days postflight, the number of neutrophils expressing CD11a was comparable to that of the control subjects.

CD11b (Mac-1): CD11b is the most abundant ß2-integrin on the surface of neutrophils and is essential for neutrophil adherence to endothelium, chemotaxis, and diapedesis as well as phagocytosis of opsonised particles. More than 95% of the neutrophils of all the control subjects and crewmembers expressed CD11b at all three time points of sample collection (data not shown). There was no significant variation in the expression of CD11b on the neutrophils of control subjects (Fig. 4), but there was a

significant variation in the expression of CD11b on the neutrophils of all the crewmembers ($p=0.005$ for 5-day mission, $p<0.001$ for the 9-, 10- and 11-day missions). The neutrophils of the crewmembers of the 9-, 10- and 11-day mission expressed normal levels of CD11b preflight and at landing, but there was a sharp decline in the expression of CD11b 3-days postflight ($p=0.001$to 0.003).

CD11c (integrin ax): Also called the p150, CD11c is a ß2-integrin involved in the chemotaxis and phagocytosis of neutrophils. As for CD11b, more than 90% of the neutrophils of all the control subjects and crewmembers expressed CD11b at all three time points of sample collection (data not shown). There was a significant variation ($p<$ 0.001 or = 0.003) in the expression of CD11c at different timepoints among the crewmembers of all missions (Fig. 4). The expression of CD11c was lower 3-days postflight than at 10 days before flight for crewmembers of 9-day ($p=0.002$) and 10-day missions ($p=0.002$) but higher for crewmembers of the 5-day mission.

L-Selectin: It is an adhesion molecule on the surface of neutrophils involved in rolling of neutrophils on the endothelial surfaces. The number of neutrophils of control subjects (Fig.4) expressing L-selectin did not change significantly between the 3 different time points of sample collection. For the 5-day mission there was a significant decrease in L-selectin expression at R+0 whereas, for the other missions, there was a significant increase at R+0.

CD36: For the crewmembers of the 5-day mission, we saw significant changes in the number of neutrophils expressing CD36 at the three sampling time points (Fig. 4), but there was variation in the expression of CD36 among the crewmembers of the 10-day and 11-day missions.

DISCUSSION

This is the first investigation of neutrophil phagocytic capability and related functions in astronauts before and after space flight. The data presented here indicate that, in contrast to control subjects whose numbers of neutrophils and functional responses of each neutrophil do not vary over the time period covered, up to 27 days, crewmembers of space shuttle flights experienced significant changes in both of these parameters. Some of these changes may be attributable to stress, others to the effects of microgravity for up to 14 days. In the absence of good models to simulate these situations separately, it is difficult to assign the specific cause of the observed changes to just one of these effectors.

Our current data are consistent with the previous observations (1, 8) that the number of neutrophils is highest immediately after landing for shuttle spaceflights of 2-11 days duration. Similar changes have also been reported for the Russian Salyut flights (10). In view of the reported elevated cortisol and other stress hormones (110, such an increase may be attributable to release of demarginated neutrophils (12-13). As we see a single peak in the functional responses of the neutrophils of crewmembers, i.e. oxidative burst and degranulation, we conclude that, if there are demarginated neutrophils, their ability to respond functionally does not differ from the circulating non-demaginated ones. As we see a single peak in the functional responses of the neutrophils of crewmembers, we can say that the demarginated and non-demarginated neutrophils have the same functional capabilities. Unlike the report of Stowe et al. (8), neutrophil bands were only observed in 1 of the 25 astronauts in the current study,

suggesting the observed increases in neutrophils did not result from the release of neutrophils from the bone marrow.

In the absence of in-flight data it is impossible to know precisely when the cortisol level increased, i.e. during the flight or during the stress of landing. However, it is, interesting that, while the number of neutrophils is highest at R+0, the efficacy of phagocytosis by each of these neutrophils is generally highest immediately after landing (R+0), and the efficacy of phagocytosis by each of these neutrophils is significantly below that of the controls. Phagocytosis has not previously been studied in astronauts, and comparisons to existent literature hence cannot be made. The phagocytosis assay used here evaluates phagocytosis via both the complement and the Fc receptors while our other functional assays determine only oxidative burst and degranulation mediated via the Fc receptors. It is known that the mechanism of this mediation is different for these two neutrophil effector functions, a fact which is reinforced by the present findings. The effects of space flight on each of these properties therefore cannot be expected to be directly comparable and interdependent. However, the trends are clearly similar, indicating, with only one or two exceptions among the 25 crewmembers, lower than control functional responses by each neutrophil even though its receptor expression (e.g., Fc receptors CD16 and CD32) was not statistically decreased.

For the present studies oxidative burst was evaluated by measuring the oxidation of an immune complex-linked reduced fluorescein within the phagocytic vacuole. As indicated above, none of the control subjects exhibited large differences between the three days on which their oxidative capability was measured, and 7 of the 9 controls showed a remarkably equal capability as well when one considers the normal variation

to be expected between individual donors. In contrast to controls, among the crewmembers there was a marked effect of the day on which their blood was tested, with the greatest effect being noted in the entire crew of the shortest flight and one crewmember (#1) of the 10-day mission. Except for the latter individual, the oxidative burst was greater immediately upon landing than 10-days before launch. The 3-day time span to the next sampling yielded mixed results. It is, however clear, as shown in Fig. 2, that the general effect of spaceflight and its pre- and post- flight stresses is to decrease the ability of the crewmembers' to oxidatively attack and destroy foreign entities entering their blood stream. The decrease was, in some cases, greater than 60% and was still evident 3-days after landing, a time when the individual's available neutrophil pool (i.e. number of neutrophils) had already decreased to near normal values from the 85% (average) increase observed immediately after landing. This could mean that returning crewmembers are, for a period of time greater than 3-days, more susceptible to infections than they had been long before launch. This effect becomes more pronounced with increasing mission duration.

In the studies reported here degranulation was evaluated via elastase release into the phagocytic vacuole, using an immune complex-linked elastase-specific coumarin substrate, which becomes fluorescent upon cleavage (14). None of the control subjects exhibited significant variations in elastase release between the three days' samplings, and 8 of the 9 had similar degranulation profiles. Since the mechanism by which oxidative burst and degranulation are mediated by Fc receptor occupancy differs, the drawing of parallels between these two effector functions of neutrophils is problematic. Thus, while the oxidative burst (Fig. 2) was lower (in 24 of 25 crewmembers) before

take-off than immediately upon landing, the same neutrophils generally exhibited a lower elastase release at landing compared to preflight values, with again a great variability at 3 days postflight. However, most of the crewmembers exhibited release comparable to controls by the time they had been back on the ground for 3-days and this was not effected by the length of the mission.

Although one would expect that the above effects of the flight preparation, flight itself, and postflight recovery were paralleled by effects on the expression of two main surface receptors involved, CD16 (FcRIII) and CD32 (FcRII), this was not true. That is, an increase or decrease in receptor expression was not mirrored by an increase or decrease in oxidative burst or elastase release (compare Fig. 2, 3 and 4).

One must therefore conclude that either the mechanism mediated by the receptors or that factors controlling the degranulation themselves, such as cytoplasmic pH, which has been shown to control elastase release (14), is altered by the combination of stress and microgravity. The extent to which these separate factors affect neutrophil function cannot be determined as yet. It is, however, clear that spaceflight alters neutrophil functions and interferes with these phagocytes' ability to dispose of invading particles, rendering astronauts potentially more susceptible to infections. This is also dependent on the length of the mission as with increasing mission duration we saw an increased suppression of the neutrophils phagocytic functions and ability to exhibit an oxidative burst. Because after a 5-day short-term space flight, the astronaut's neutrophils are capable of phagocytizing bacteria, exhibiting an oxidative burst and releasing the granular contents as well as normal subjects, but when the mission duration is

increased to 9 days or more, we saw a decrease in the ability of the neutrophils to phagocytize bacteria and release oxidative radicals.

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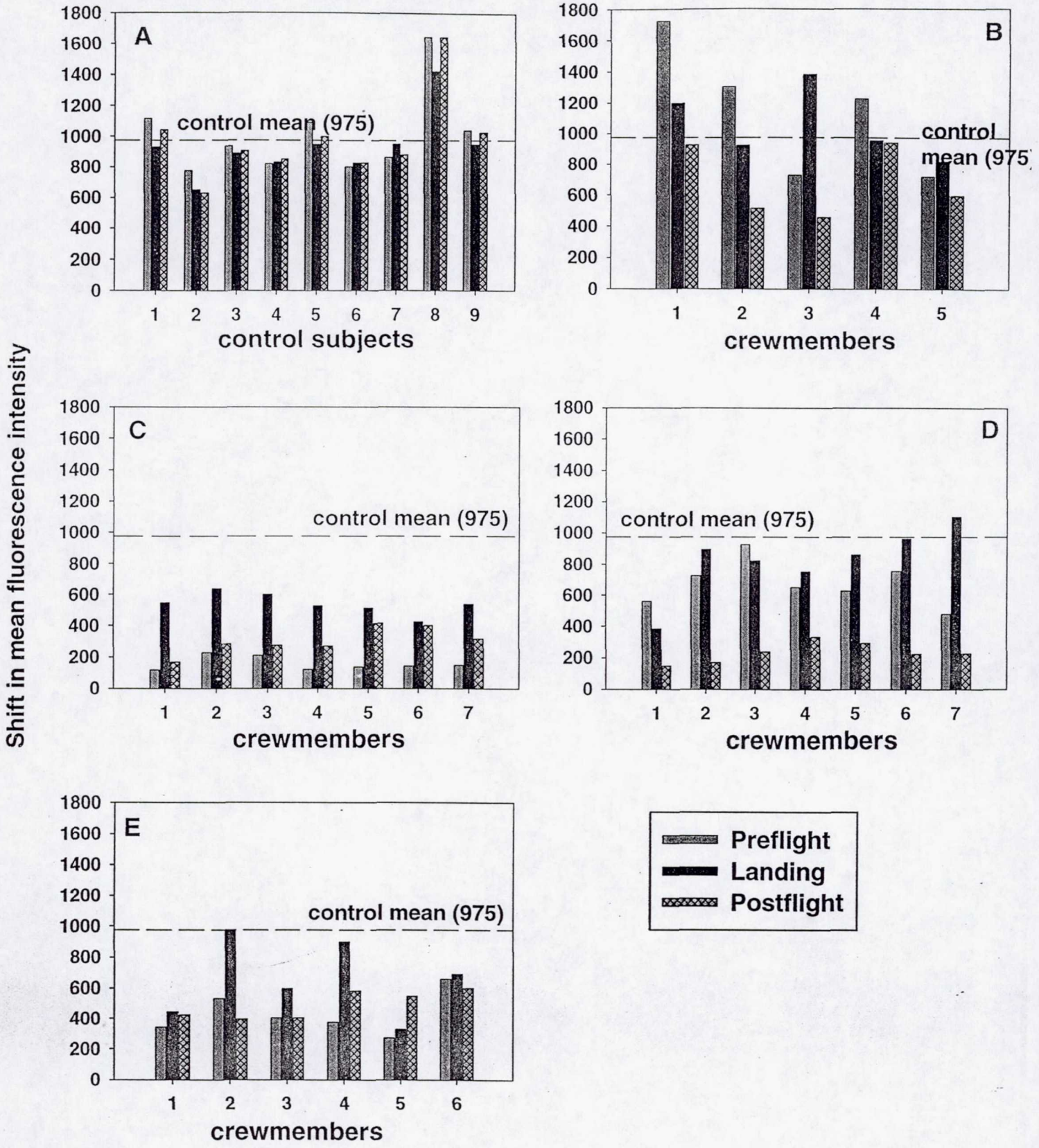
LEGENDS

Fig. 1: Phagocytosis of opsonised and heat killed *E.coli* -FITC by neutrophils of control subjects (A) and crewmembers of 4 shuttle missions (B: 5-day mission; C: 9-day mission; C: 10-day mission; D: 11-day mission).

Fig. 2: Oxidative burst capacity of the neutrophils of control subjects (A) and crewmembers of 4 shuttle missions (B: 5-day mission; C: 9-day mission; C: 10-day mission; D: 11-day mission).

Fig. 3: Degranulation of neutrophils of control subjects (A) and crewmembers of 4 shuttle missions (B: 5-day mission; C: 9-day mission; C: 10-day mission; D: 11-day mission). The 'mean control' line is the mean of all the values from all the control subjects except #6. The value for control #6 was omitted from the calculated mean value as an outlier because it lay more than 3 standard deviations away from that mean for unknown reasons.

Fig. 4. Expression of surface molecules on the neutrophils of control subjects and crewmembers of the 5-day, 9-day, 10-day and 11-day mission.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control mean (975)
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B
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C
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E
control mean (975)
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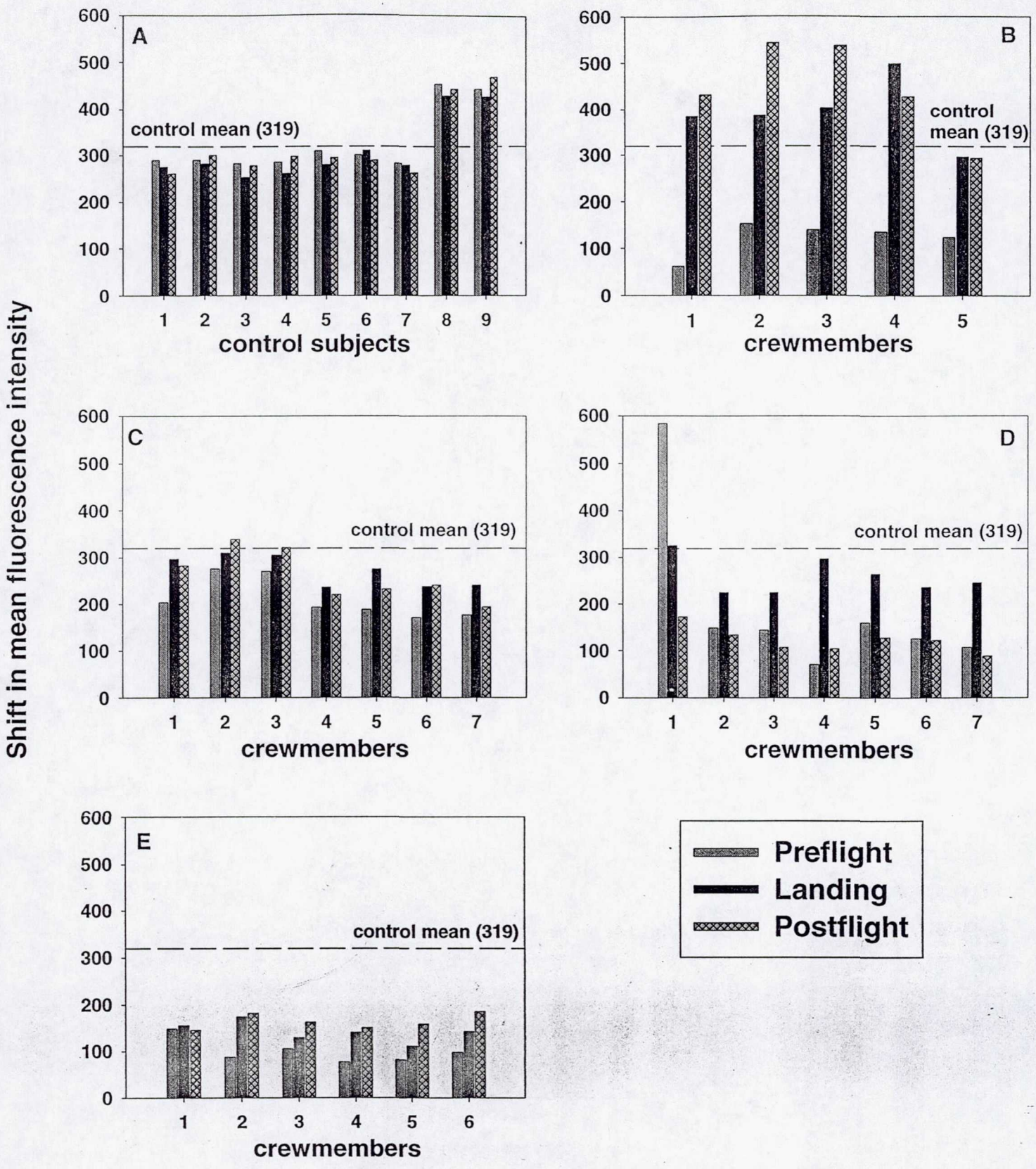
Shift in mean fluorescence intensity
Preflight
Landing
Postflight

Fig 1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Preflight
Landing
Postflight

Fig 2

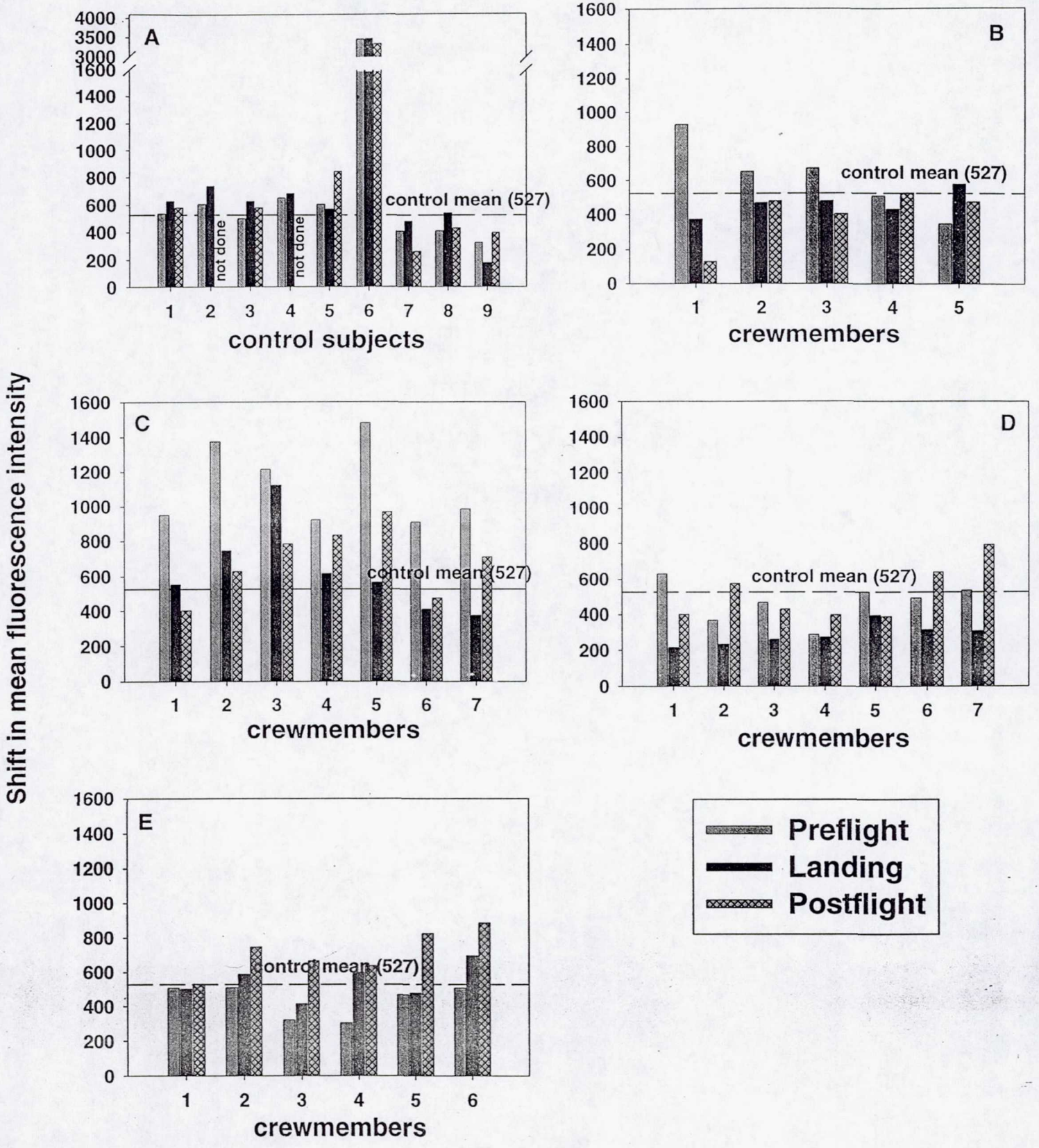
A
B
C
D
E
control mean (527)
not done
not done
control subjects
crewmembers
Shift in mean fluorescence intensity
Preflight
Landing
Postflight

Fig 3

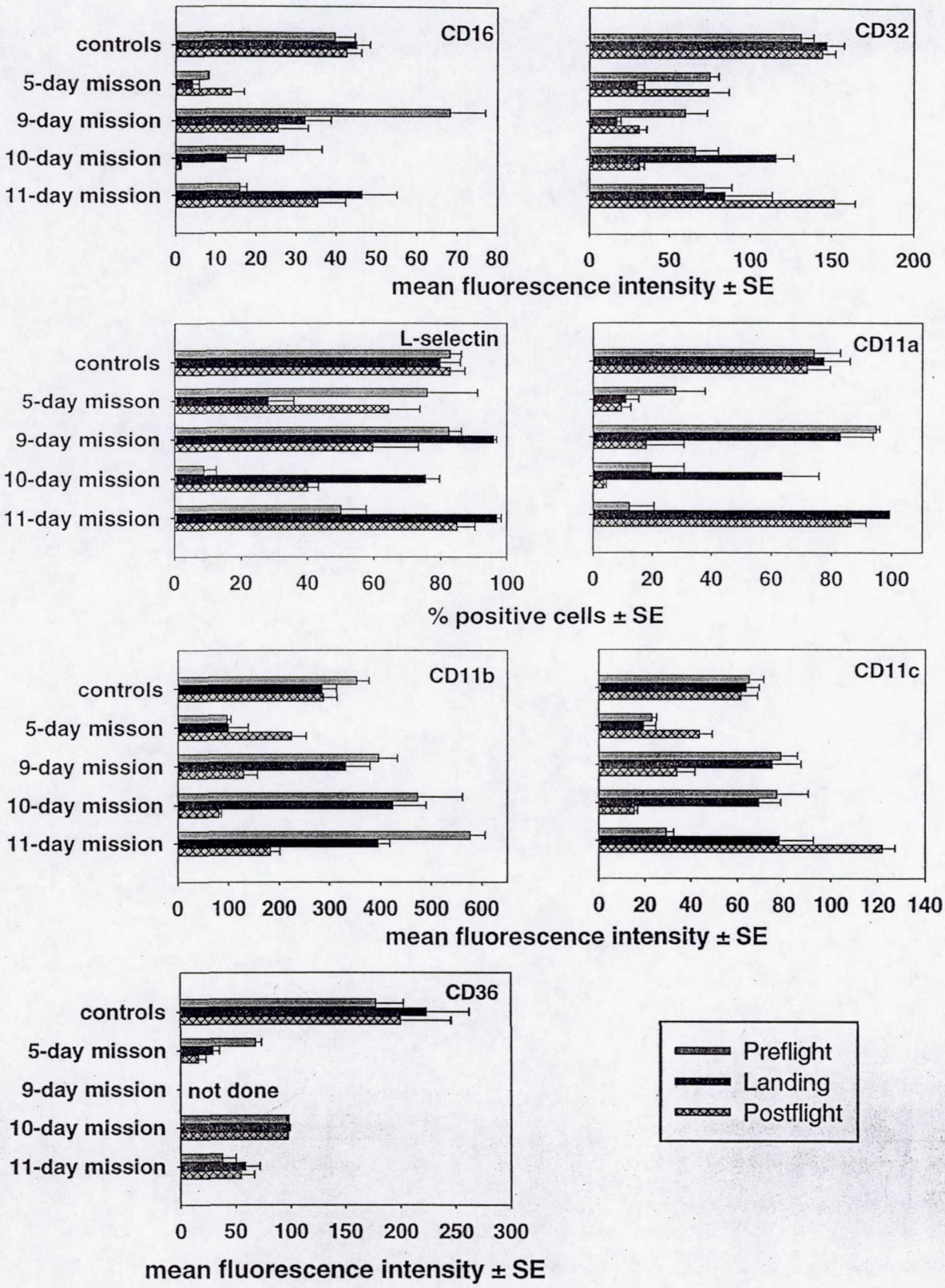
CD16
CD32
controls
5-day misson
9-day mission
10-day mission
11-day mission
0 10 20 30 40 50 60 70 80
0 50 100 150 200
mean fluorescence intensity ± SE
L-selectin
CD11a
0 20 40 60 80 100
0 20 40 60 80 100
% positive cells ± SE
CD11b
CD11c
0 100 200 300 400 500 600
0 20 40 60 80 100 120 140
mean fluorescence intensity ± SE
CD36
not done
0 50 100 150 200 250 300
mean fluorescence intensity ± SE
Preflight
Landing
Postflight

Fig 4